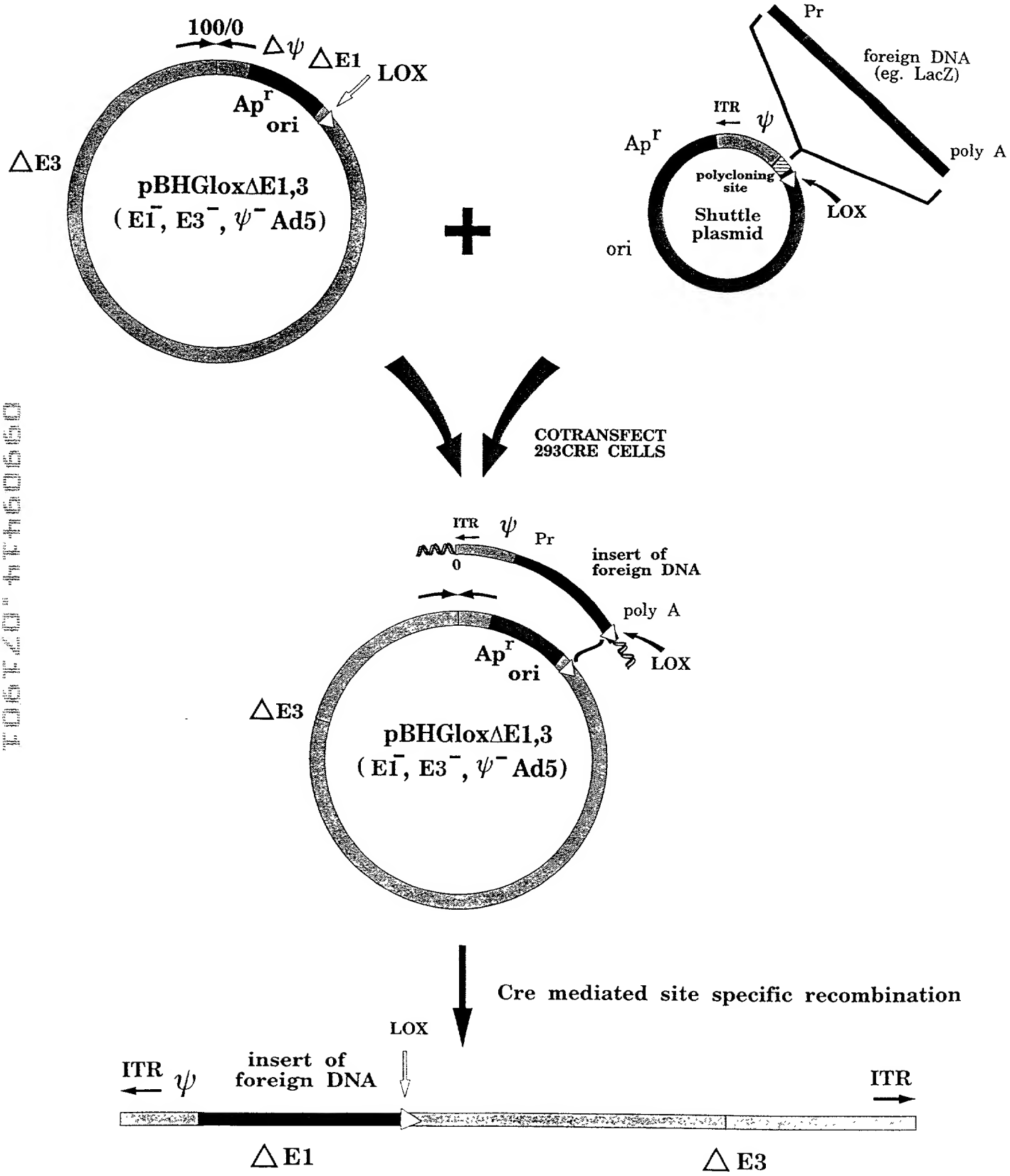
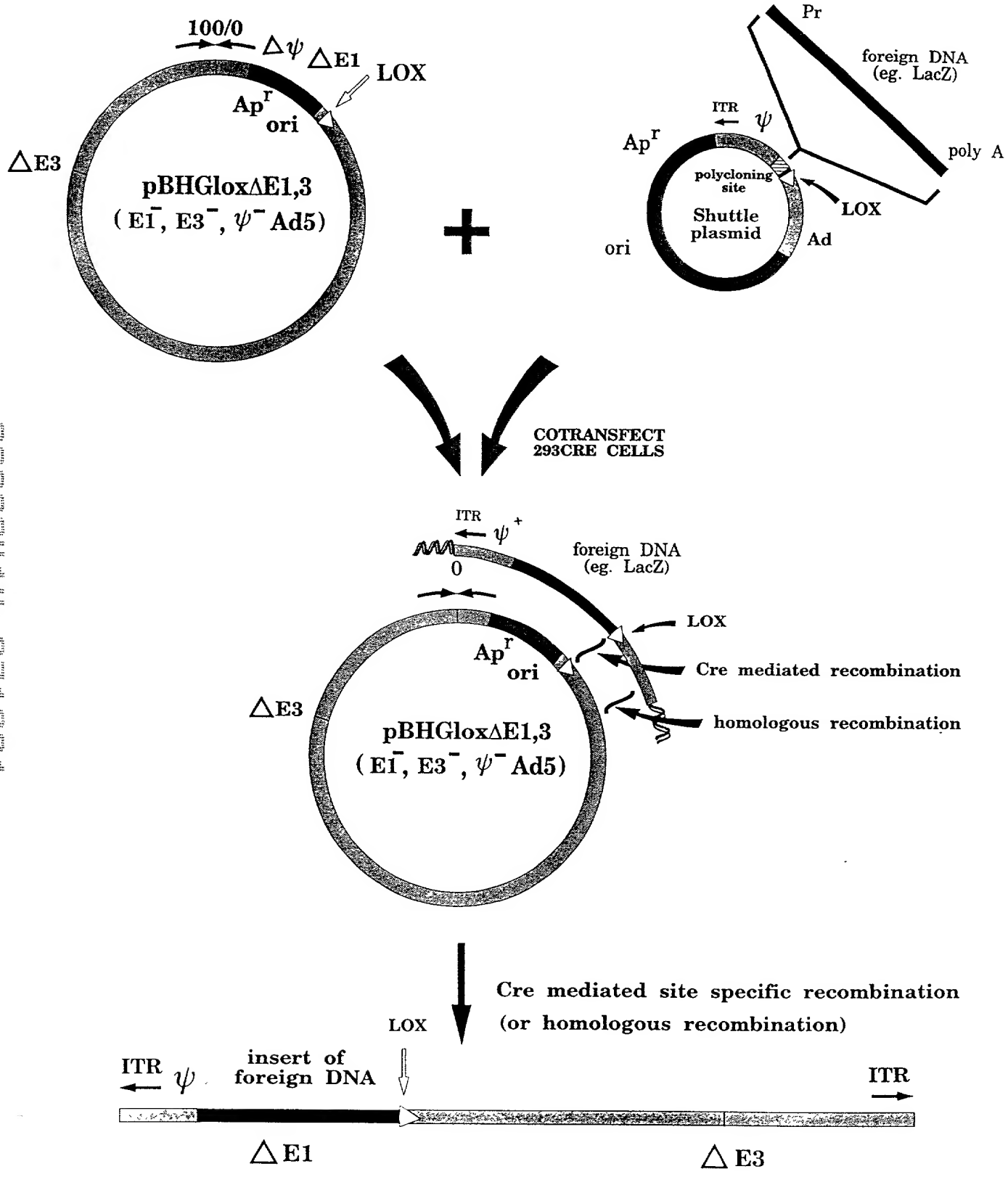
Cotransfection of 293Cre cells with pBHGloxΔE1,3 and a "Lox" shuttle plasmid for generation of Ad expression vectors



DEFECTIVE VIRAL VECTOR

Figure 1

Cotransfection of 293Cre cells with pBHGloxΔE1,3 and a "lox" shuttle plasmid for generation of Ad expression vectors



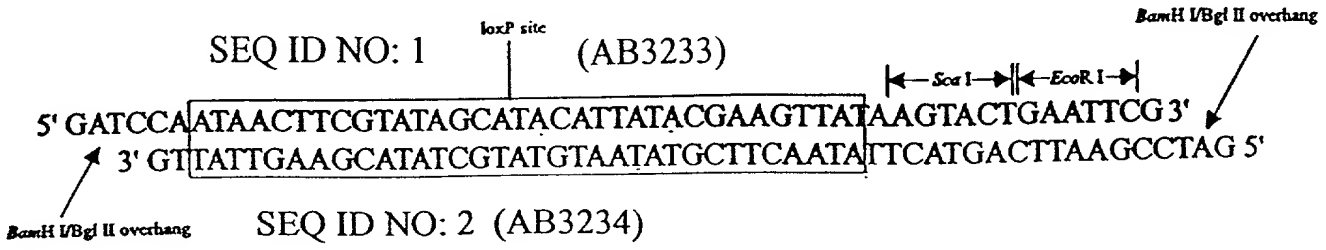
DEFECTIVE VIRAL VECTOR

Figure 2

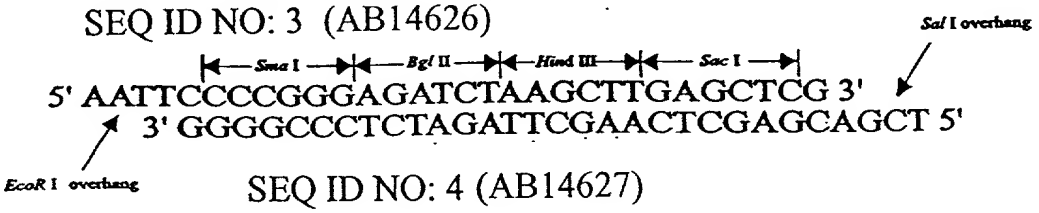
Figure 3

OLIGONUCLEOTIDES USED IN CLONING

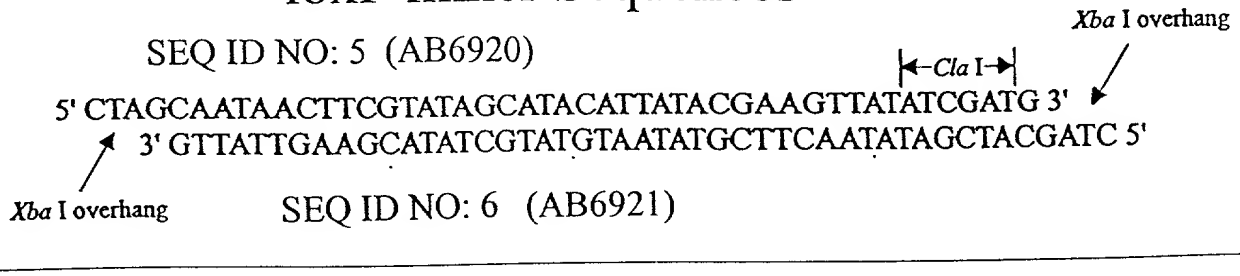
loxP linker Sequences



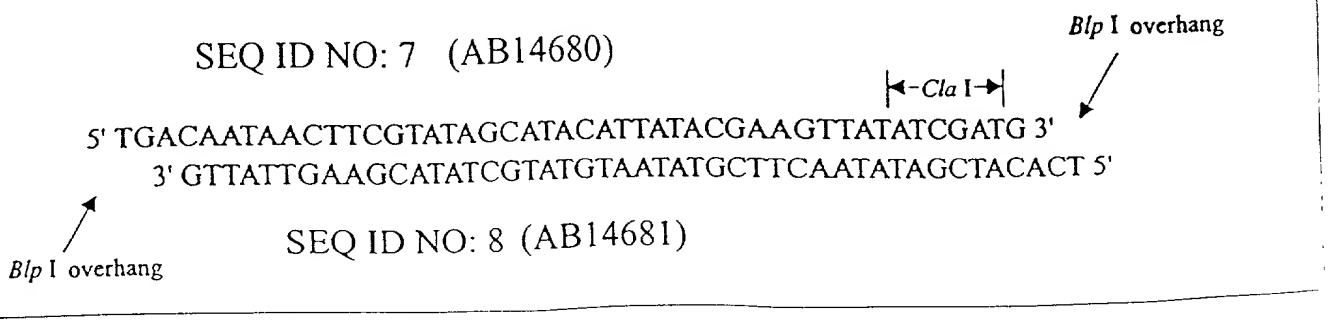
Multiple Cloning Site Sequences



loxP linker Sequences



loxP linker Sequences



CONSTRUCTION OF A CIRCULAR GENOMIC PLASMID FOR Ad VECTOR RESCUE USING THE Cre/ loxP SYSTEM

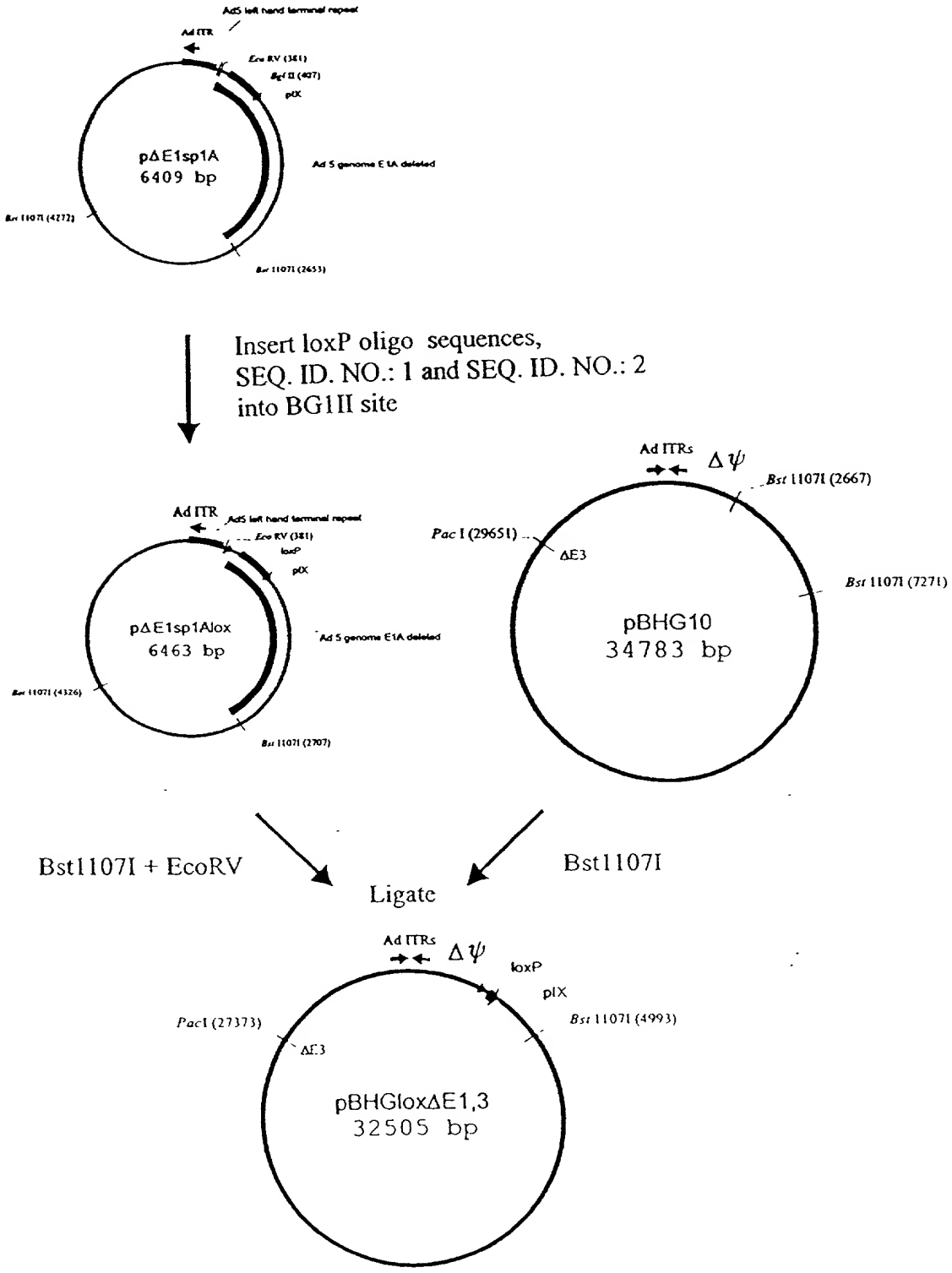


Figure 4A

CONSTRUCTION OF pBHGdX1Plox

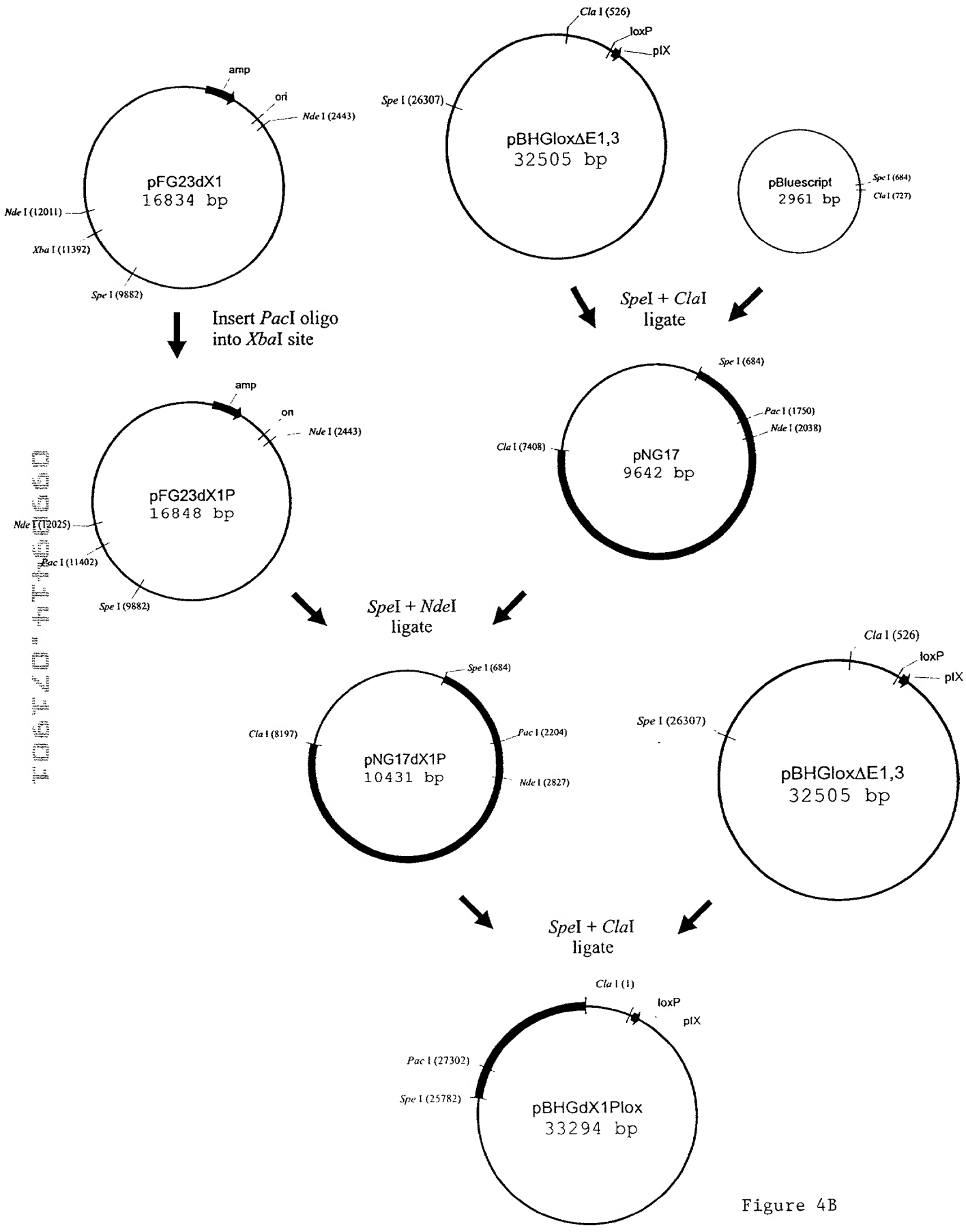


Figure 4B

CONSTRUCTION OF pBHGE3lox

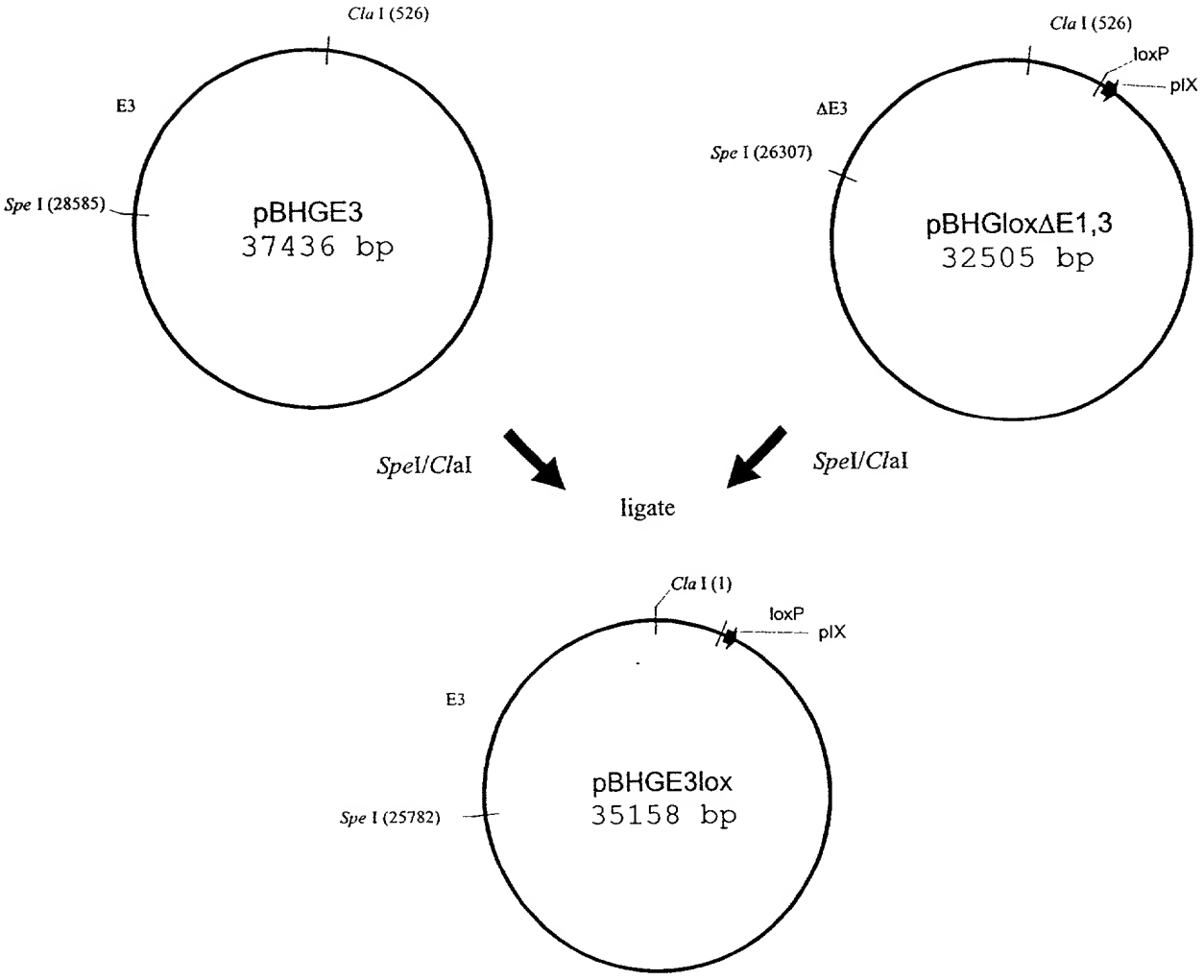


Figure 4C

CONSTRUCTION OF pΔE1SP1A & pΔE1SP1B loxP PLASMIDS FOR RESCUE OF FOREIGN DNA

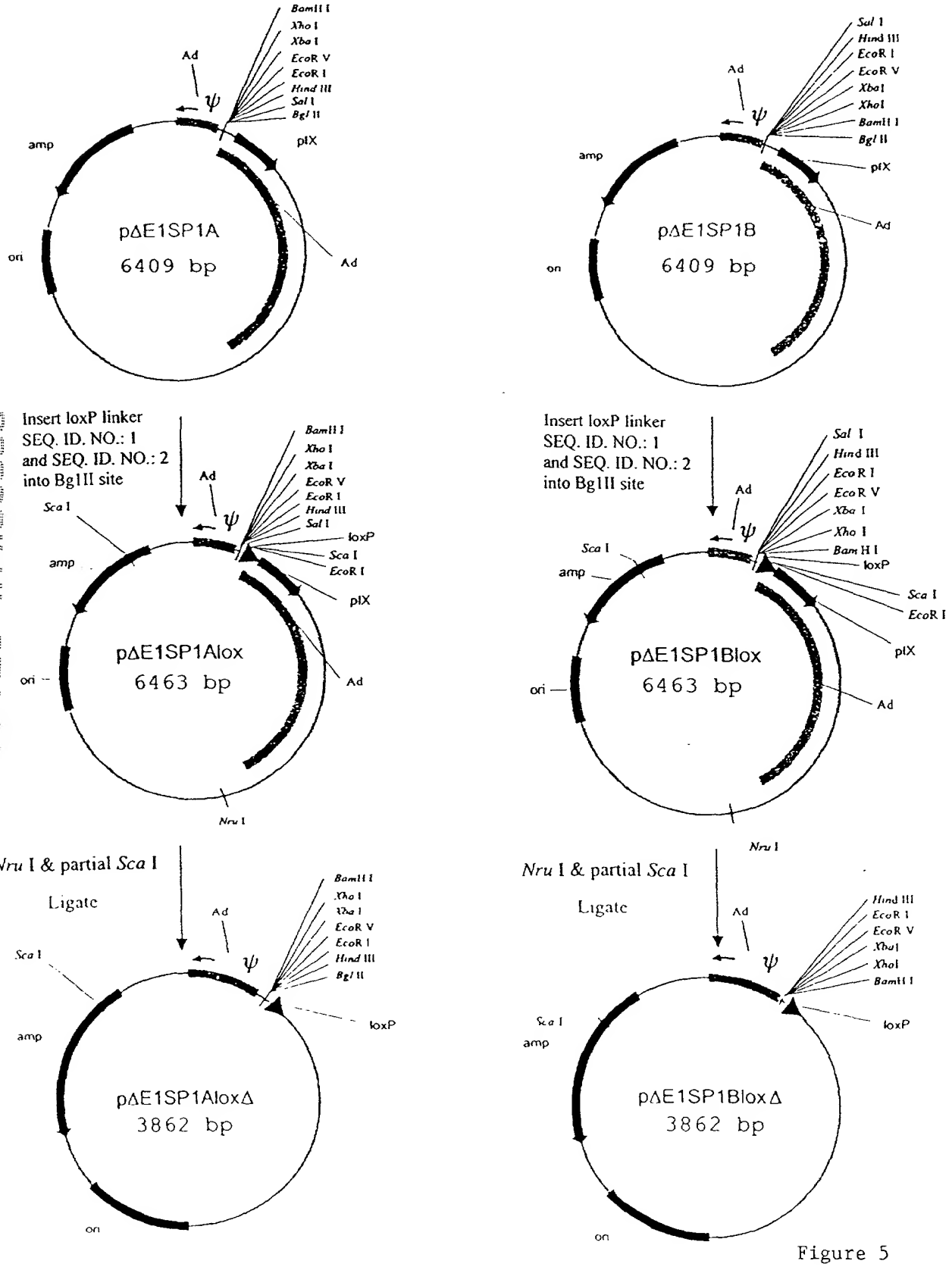


Figure 5

CONSTRUCTION OF pMH4LOX, pMH4LOXΔ and pMH4LOXΔLINK SHUTTLE PLASMIDS FOR RESCUE OF EXPRESSION CASSETTES

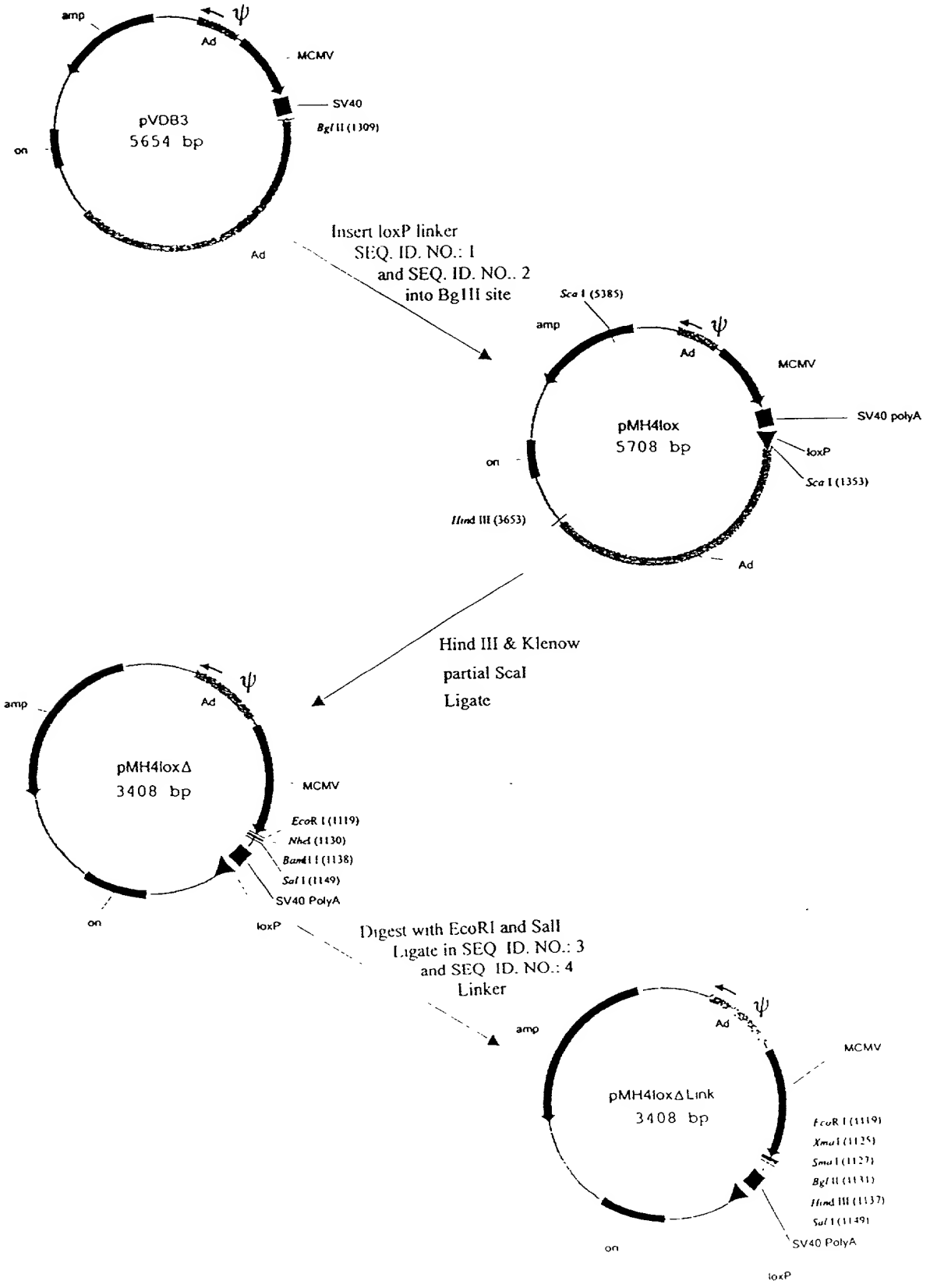


Figure 6A

CONSTRUCTION OF A SHUTTLE PLASMID CONTAINING A pUC DERIVED ORIGEN

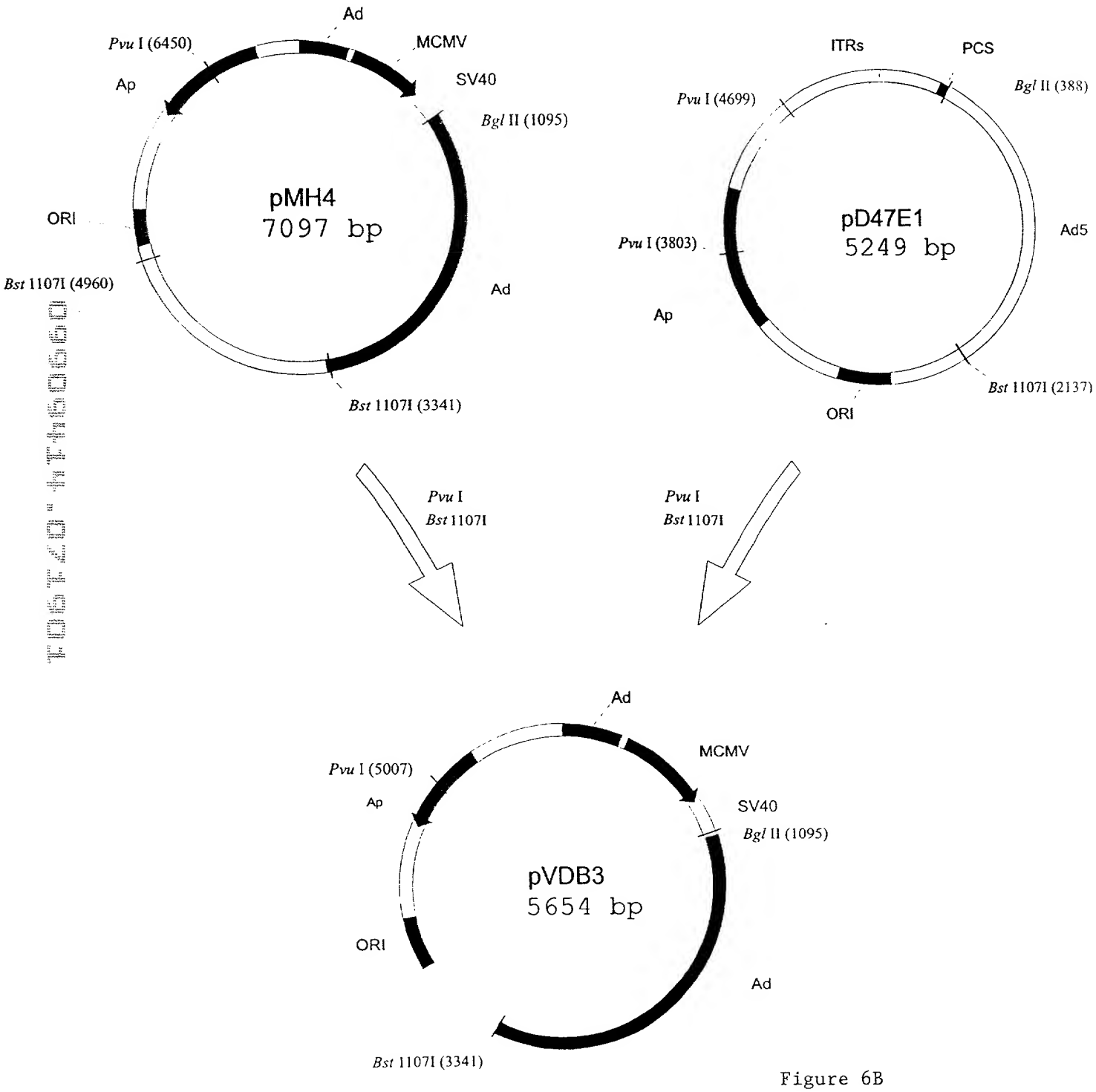


Figure 6B

CONSTRUCTION OF HCMV loxP PLASMIDS FOR RESCUE OF EXPRESSION CASSETTES

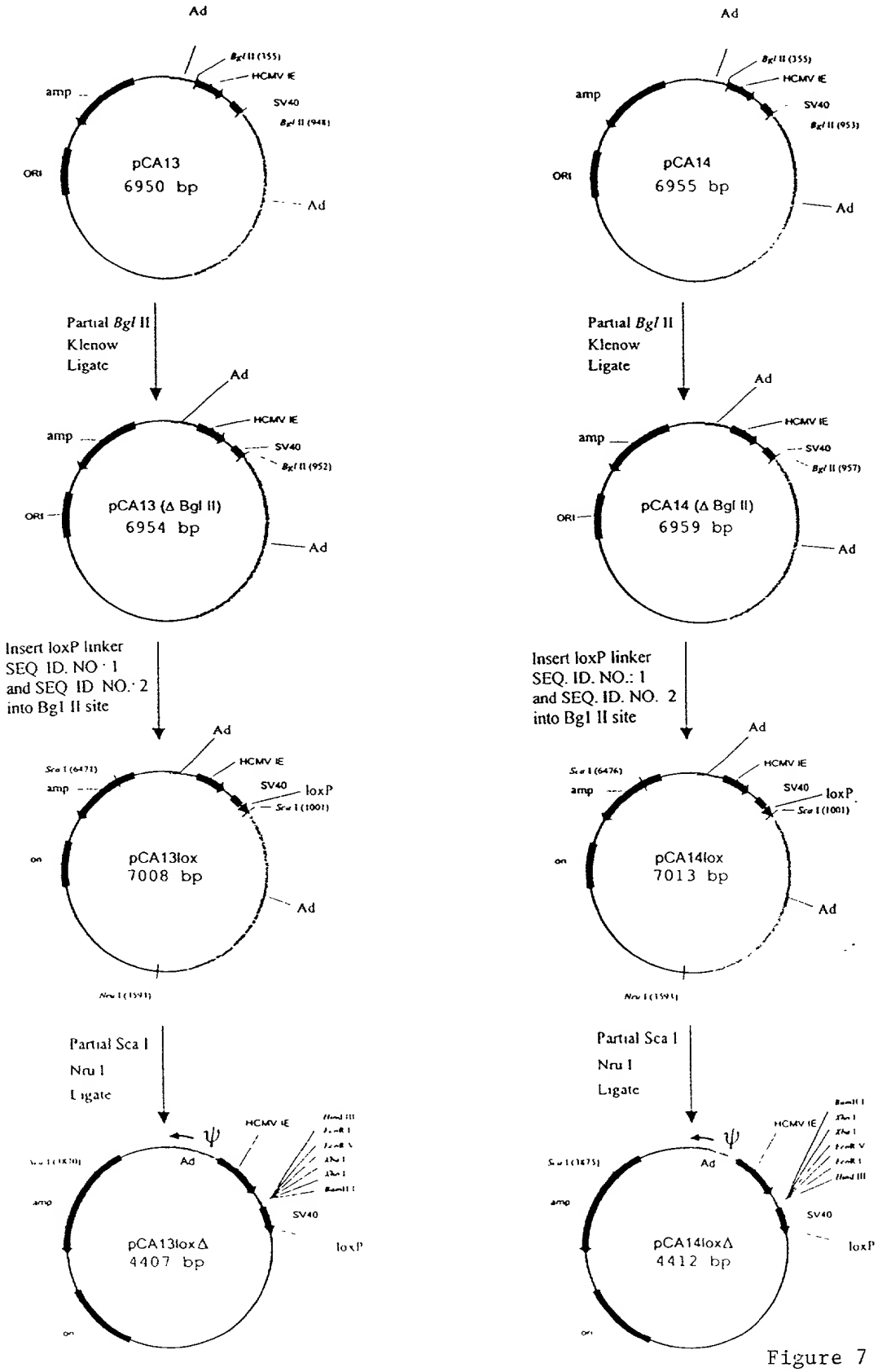


Figure 7

CONSTRUCTION OF pCA36LOX and pCA36LOXΔ SHUTTLE PLASMIDS FOR RESCUE OF LACZ

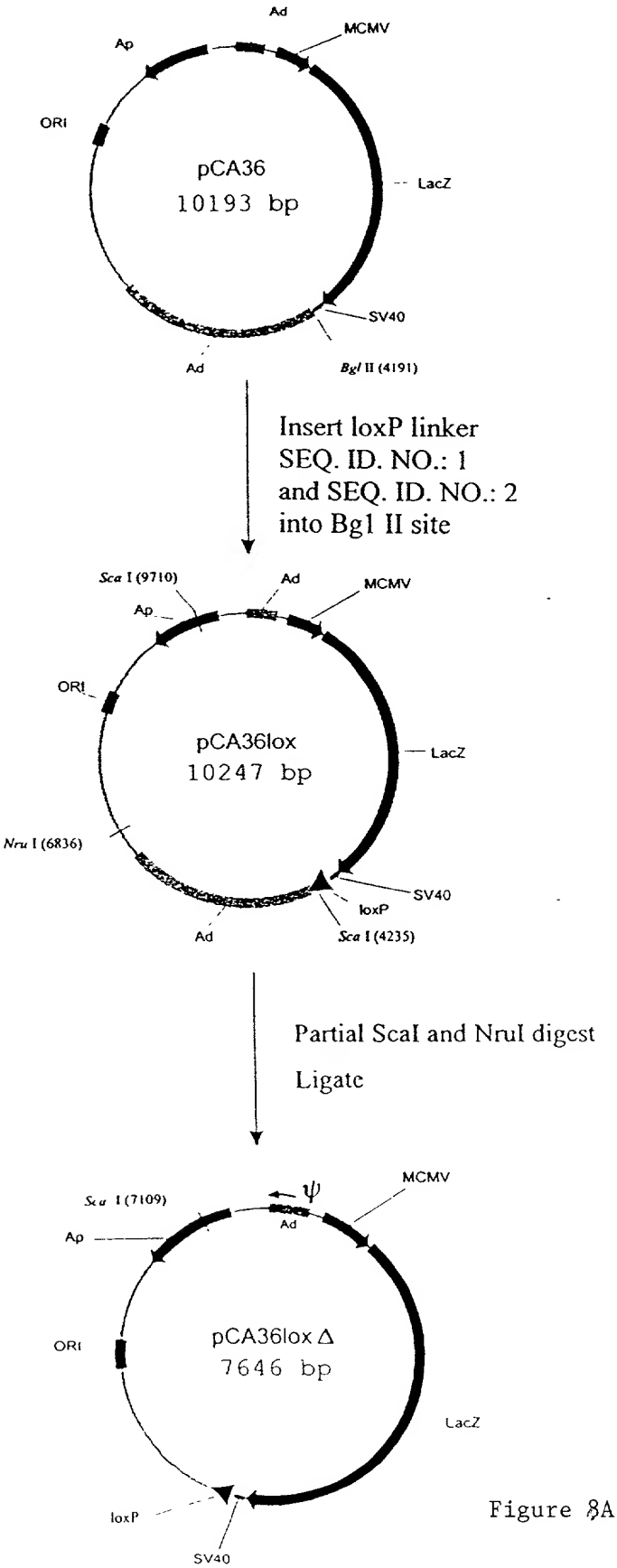
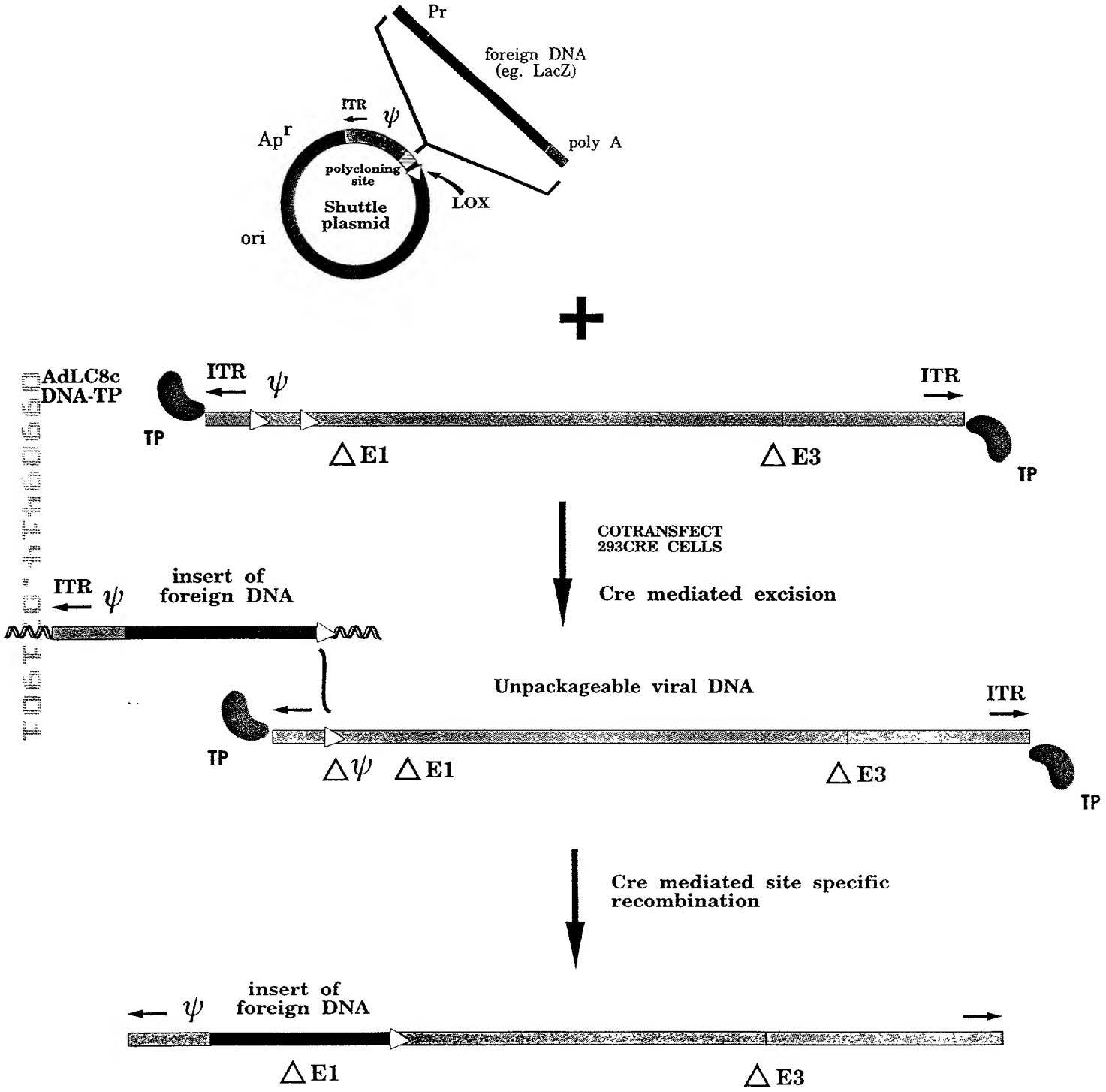


Figure 8A

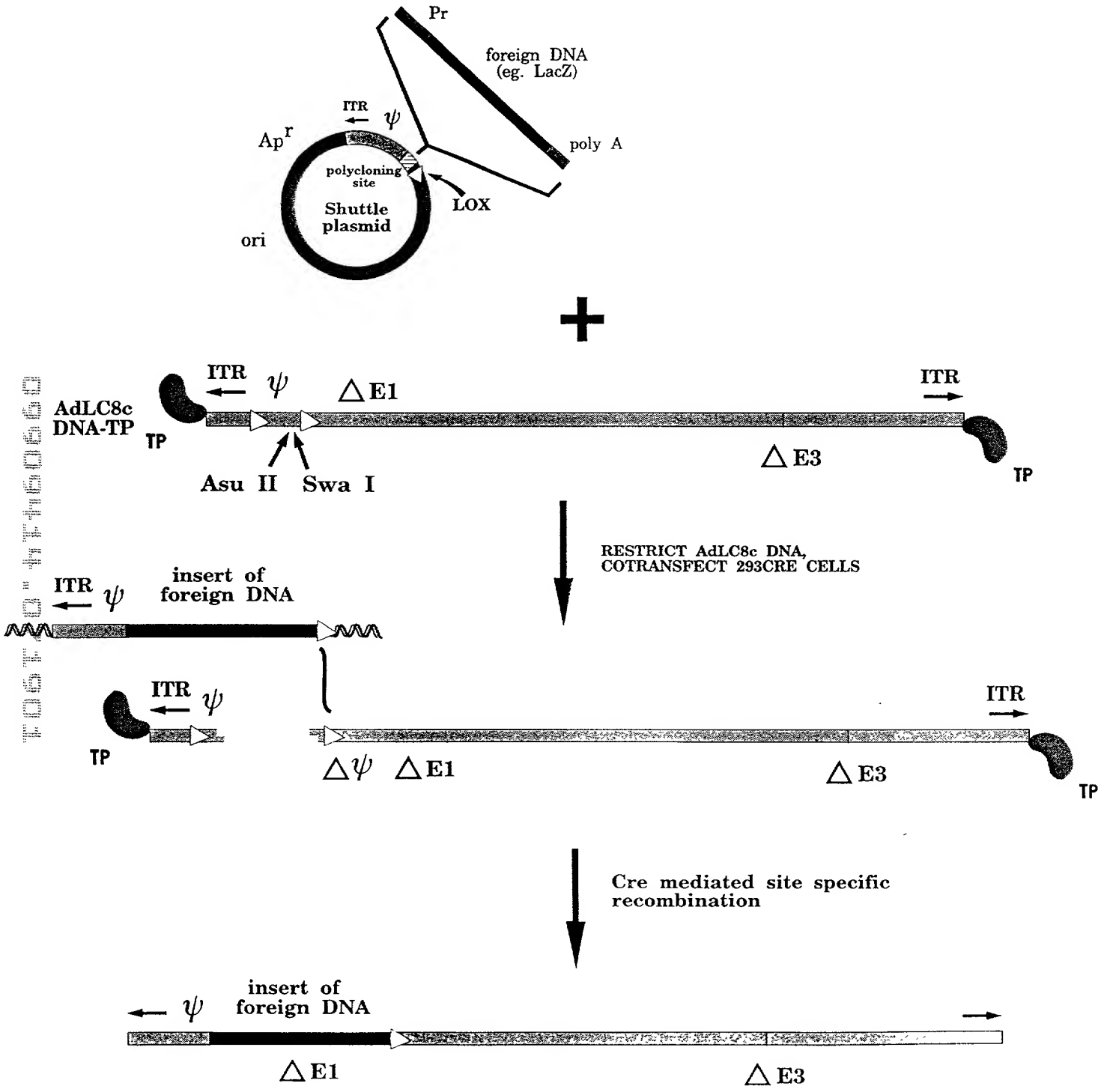
Cotransfection of 293Cre cells with AdLC8c DNA-TP and a shuttle plasmid containing a loxP site for generation of Ad expression vectors



DEFECTIVE VIRAL VECTOR

Figure 8B

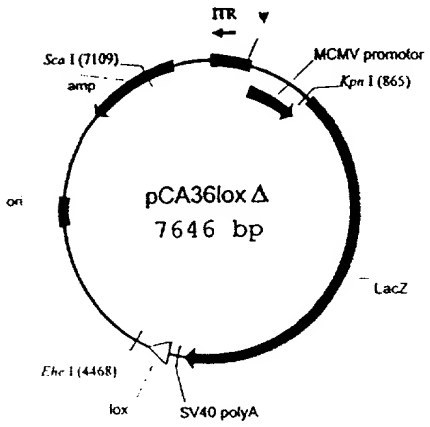
Cotransfection of 293Cre cells with restricted AdLC8c DNA-TP and loxP shuttle plasmid for generation of Ad expression vectors

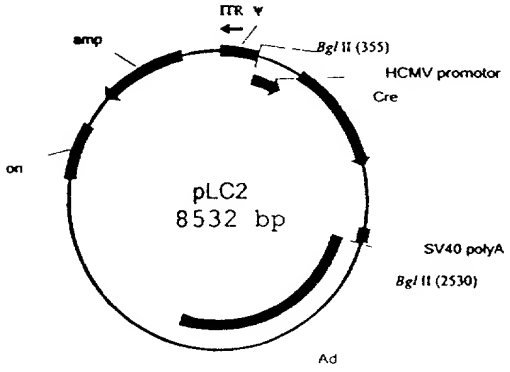


DEFECTIVE VIRAL VECTOR

Figure 8C

CONSTRUCTION OF SHUTTLE PLASMIDS EXPRESSING Cre

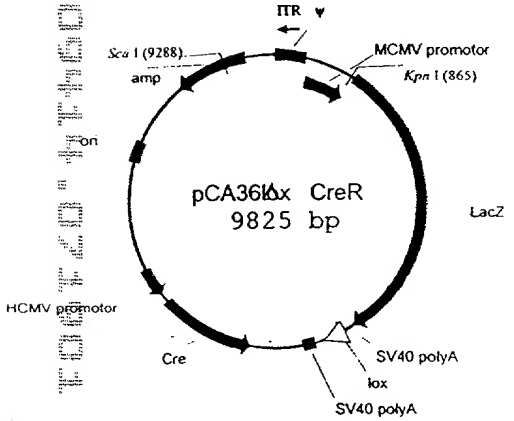




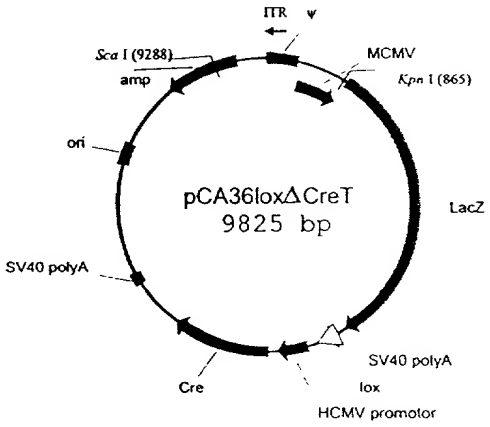
*Ehe*I

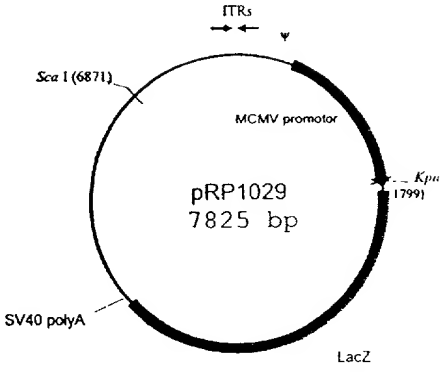
*Bgl*II, Klenow

Ligate



or





*Sca*I+*Kpn*I

*Sca*I+*Kpn*I

Ligate

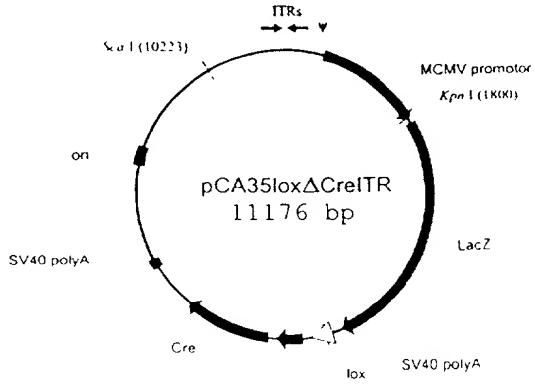
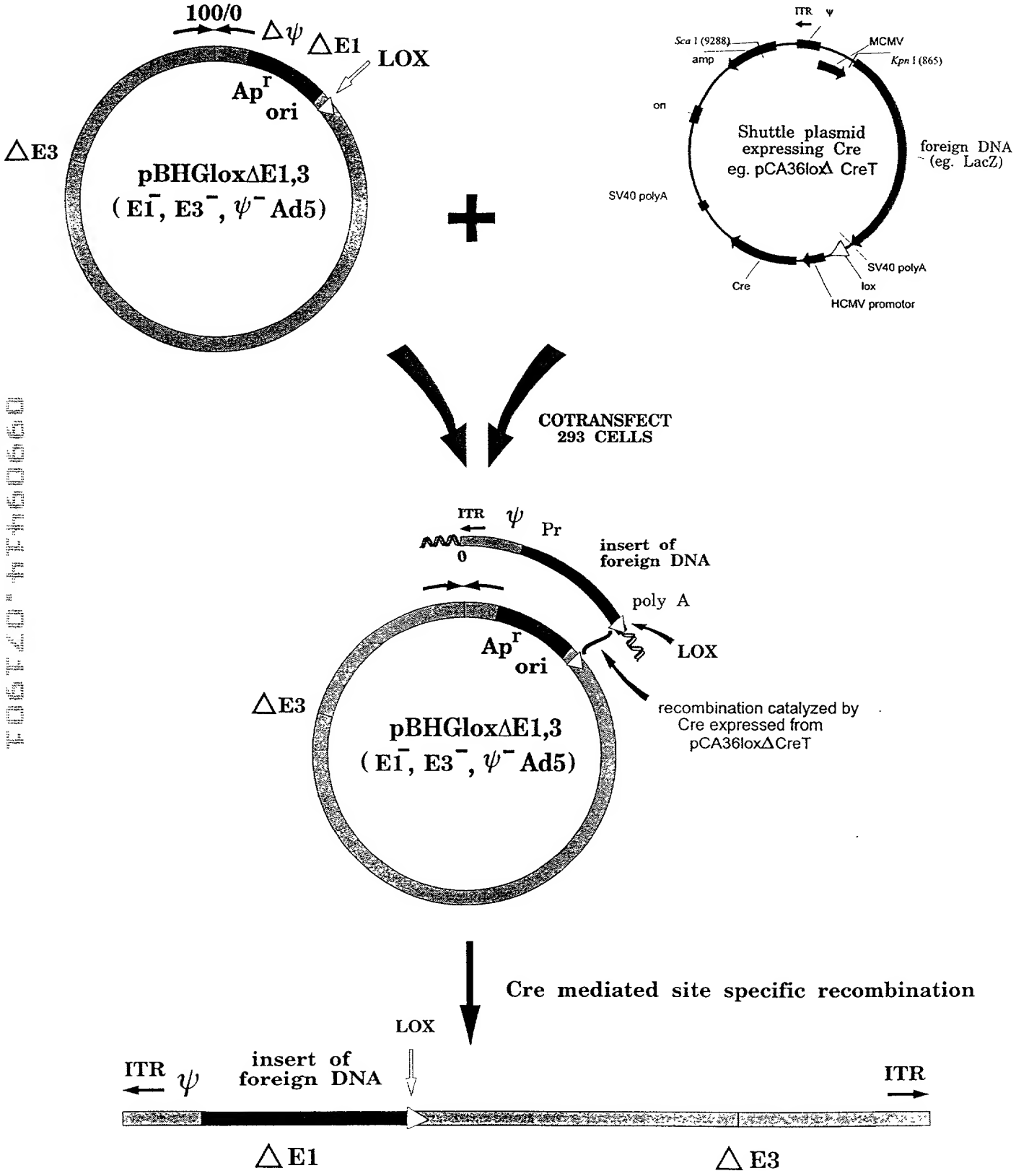


Figure 8D

Cotransfection of 293 cells with pBHGloxΔE1,3 and a "Lox" shuttle plasmid expressing Cre for generation of Ad expression vectors



DEFECTIVE VIRAL VECTOR

Figure 8E

CONSTRUCTION OF Ad GENOMIC PLASMID ENCODING CRE

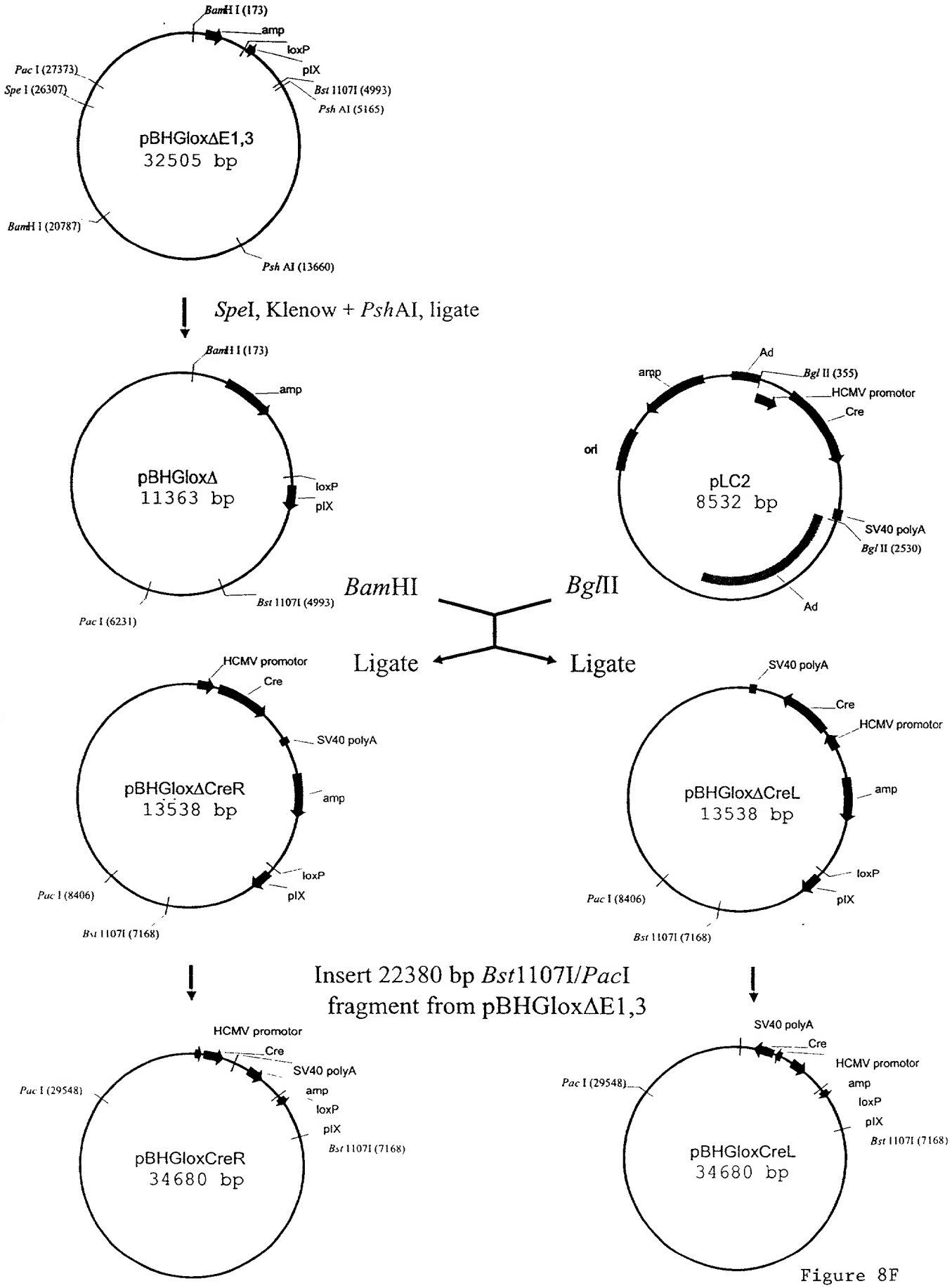
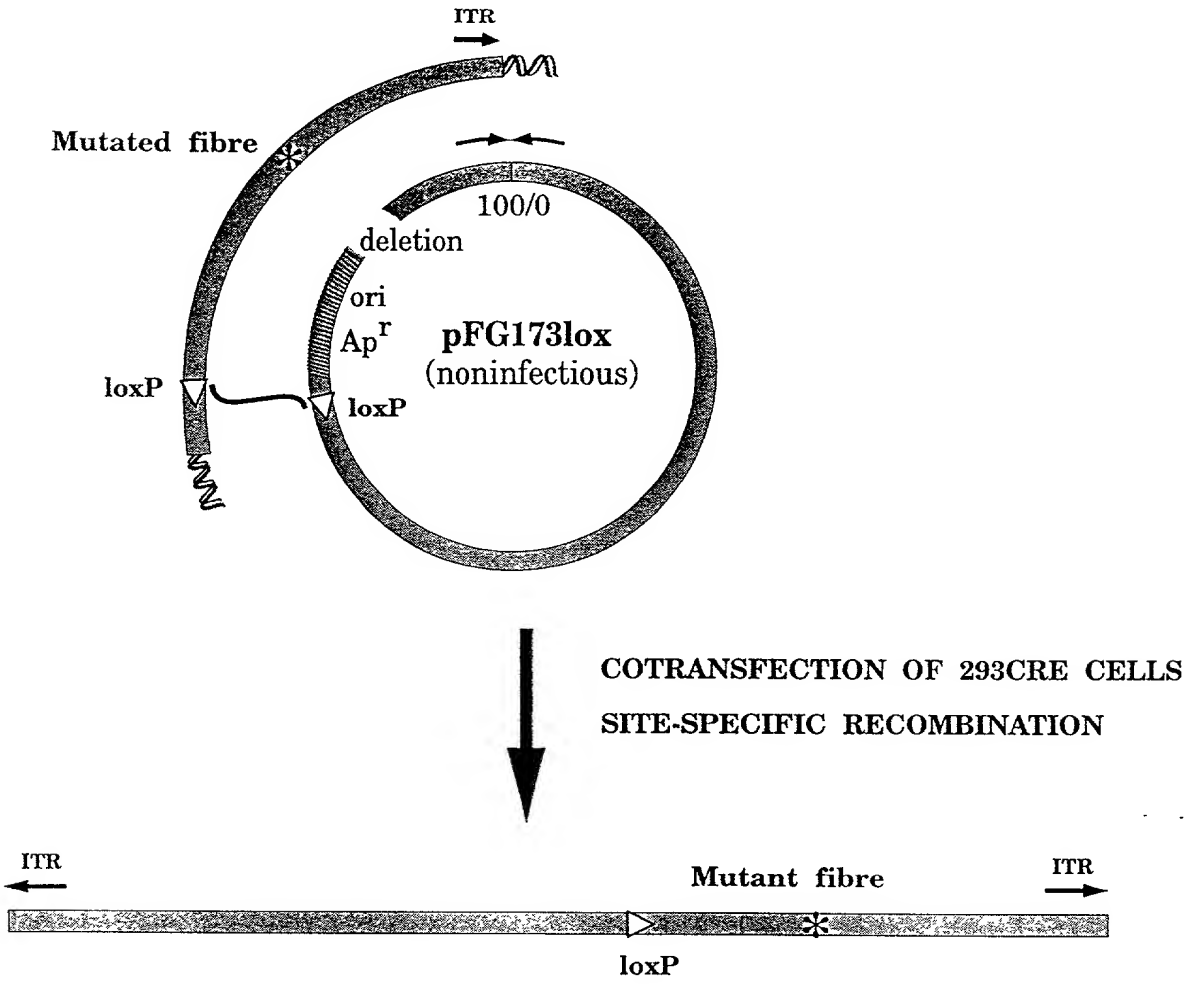


Figure 8F

RESCUE OF FIBRE MUTATIONS USING CRE/LOX RECOMBINATION



NONDEFECTIVE ($E1^+$) VIRUS WITH MUTATED FIBRE GENE

Figure 9A

CONSTRUCTION OF pAB14lox △

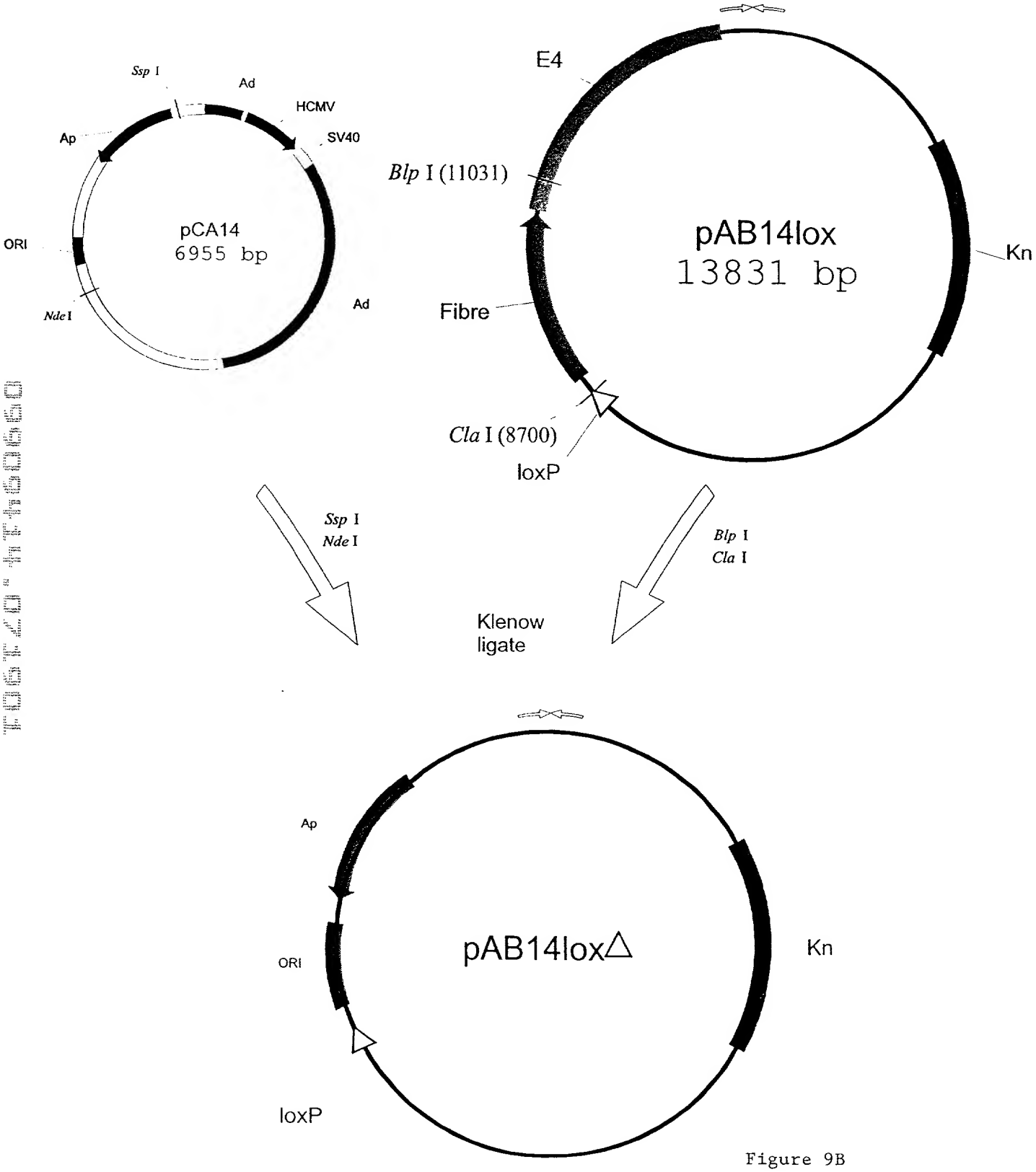
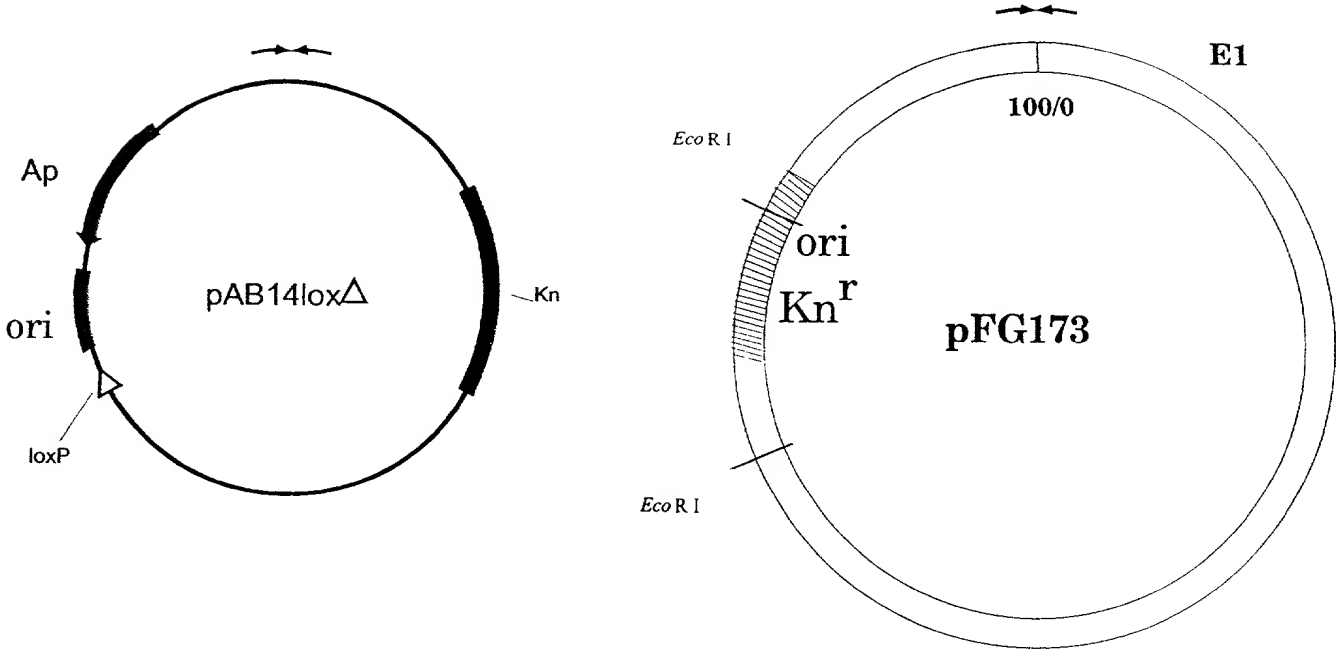


Figure 9B

CONSTRUCTION OF pFG173lox



Restriction, transformation of E. coli, homologous recombination

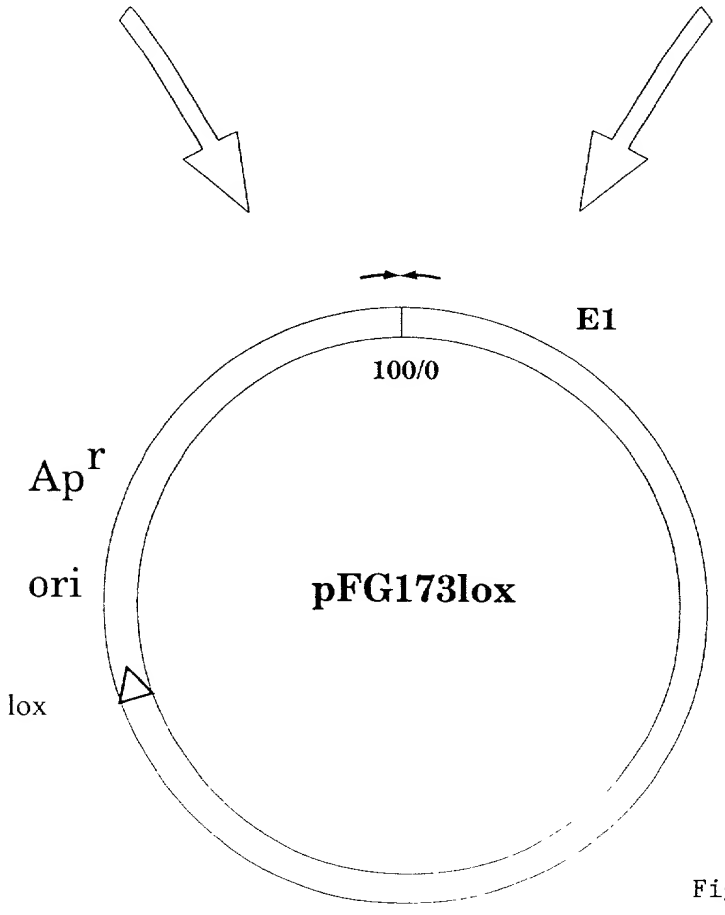


Figure 9C

CONSTRUCTION OF pFG23dX1lox AND pFG23dX1loxc FOR RESCUE OF MUTANT FIBRE INTO AD VIRUS

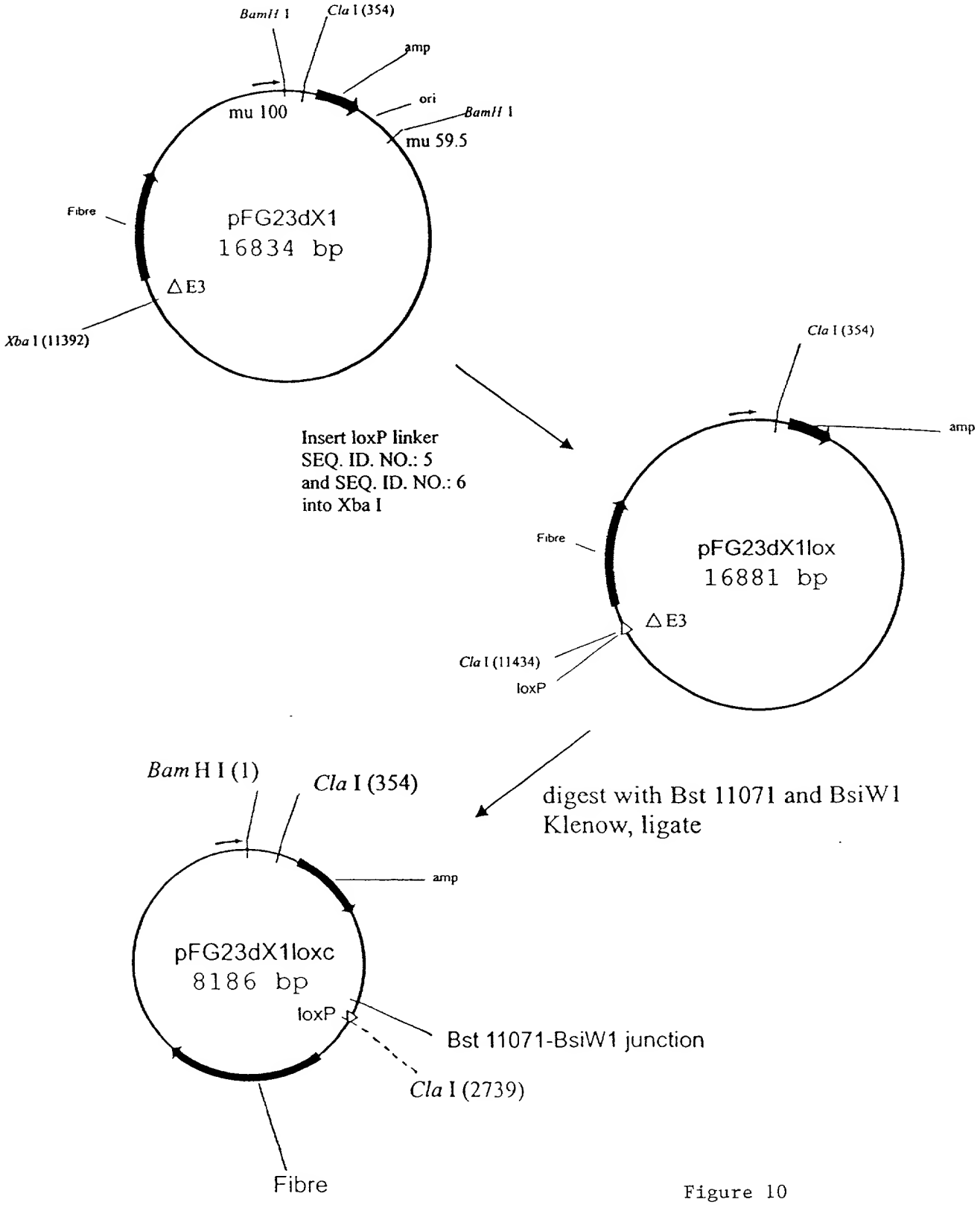


Figure 10

A PLASMID FOR RESCUE OF A FOREIGN DNA INTO AD VIRUS

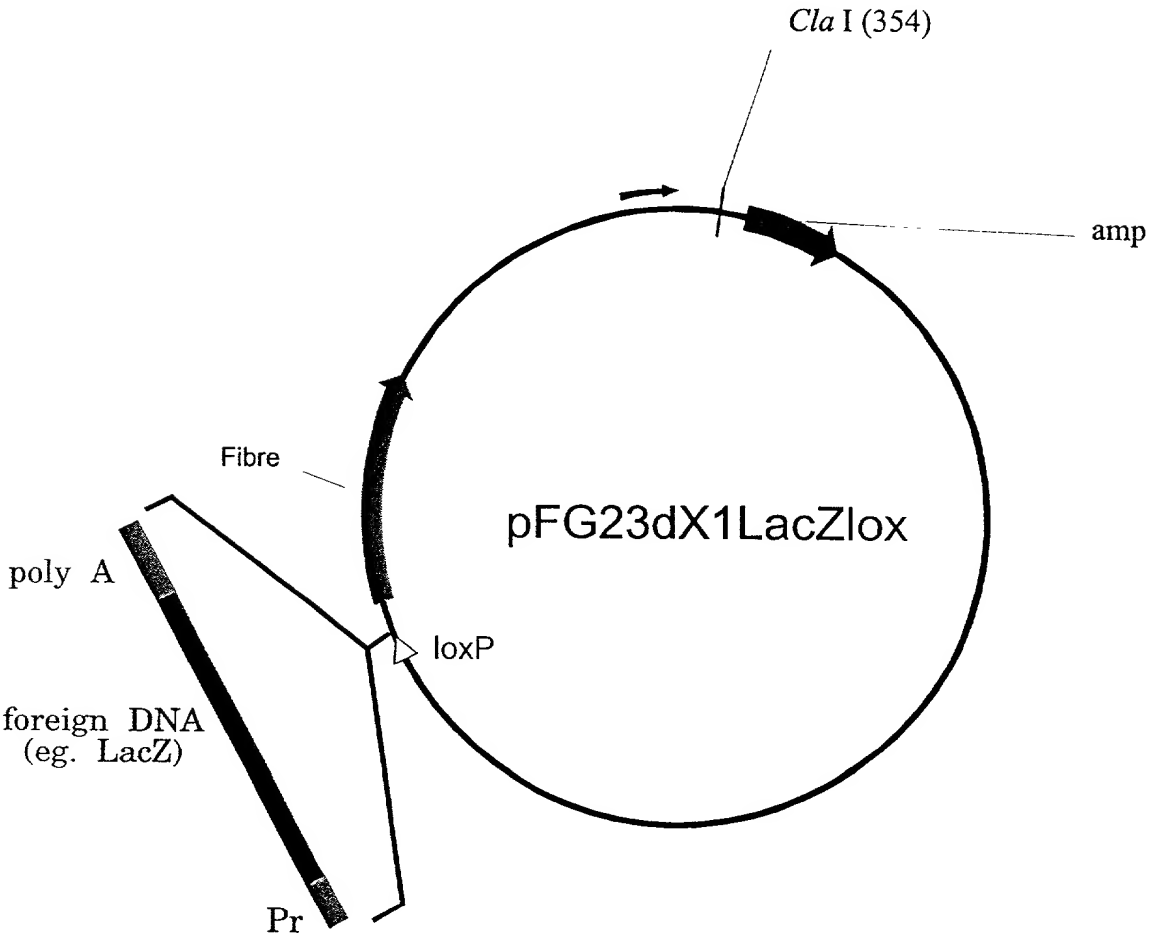
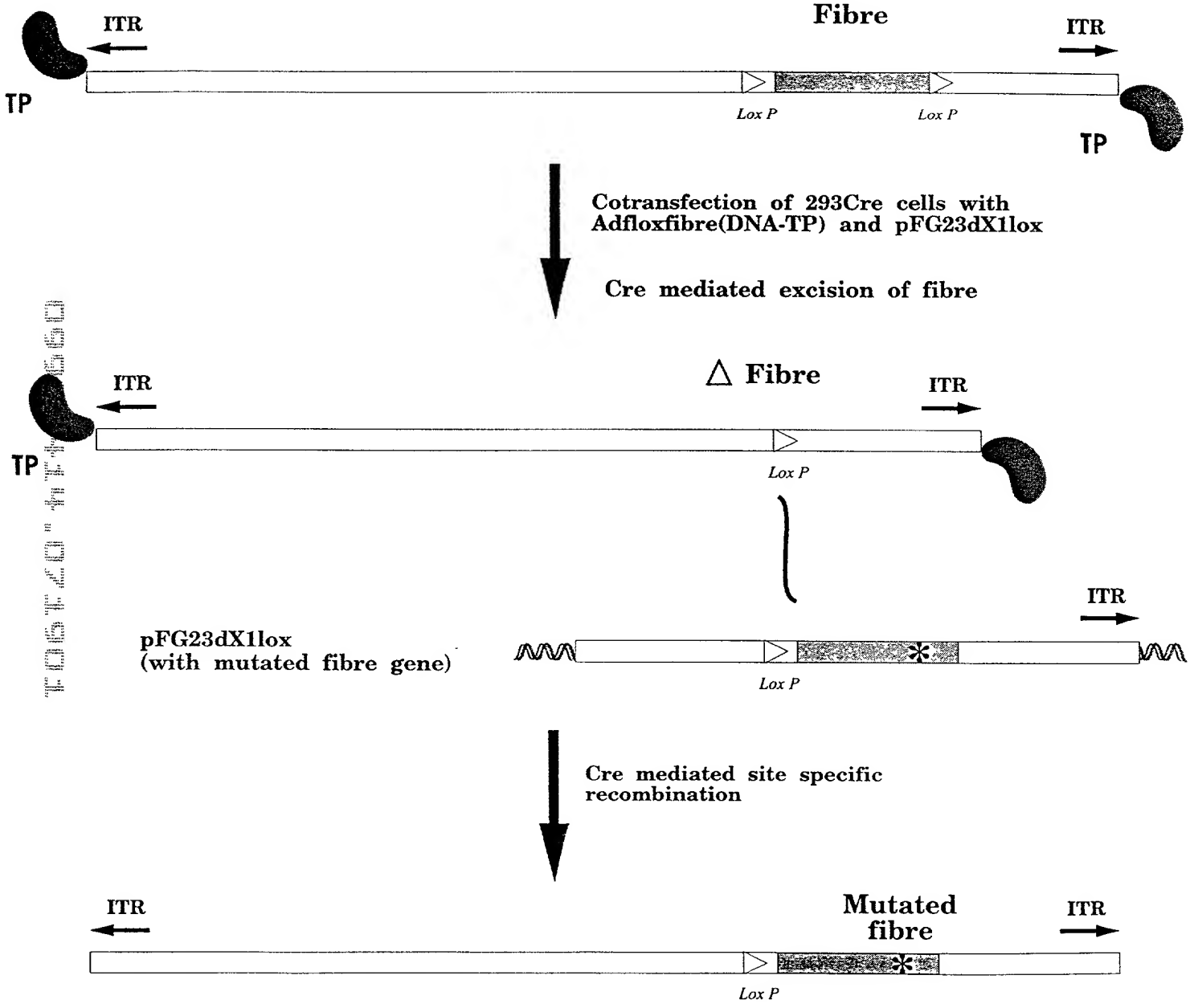


Figure 11

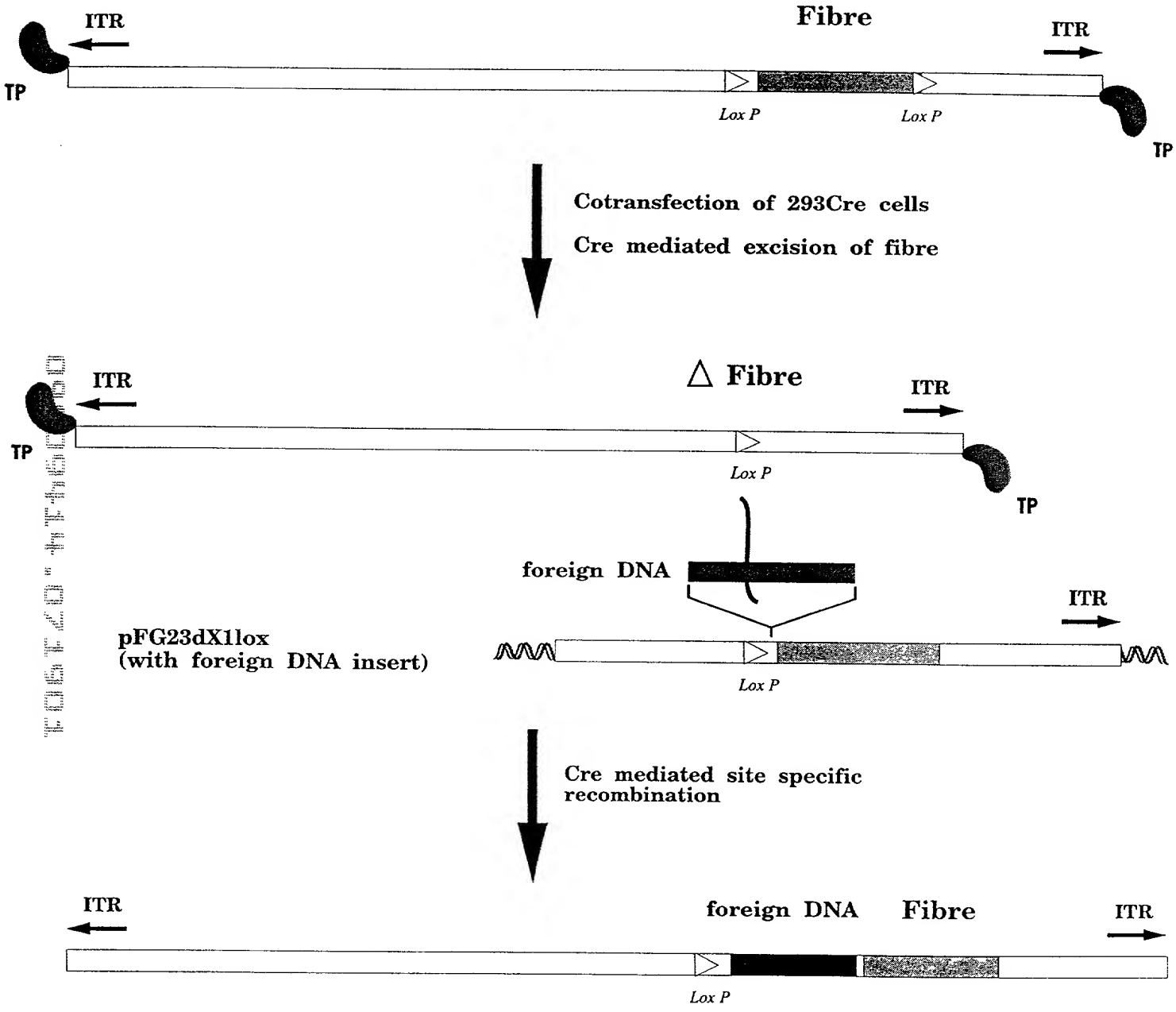
Isolation of a virus containing a mutant fibre gene by Cre-lox recombination using DNA-TP and cotransfection



RECOMBINANT VIRUS CONTAINING A MUTATED FIBRE GENE

Figure 12

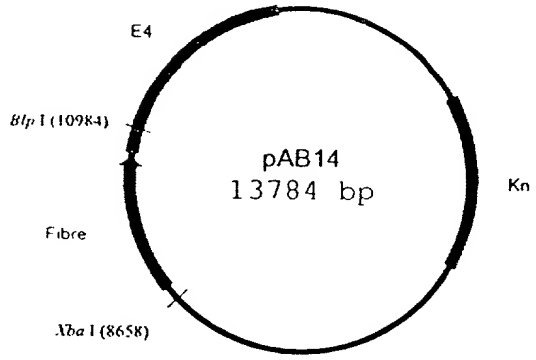
Isolation of a virus containing a foreign DNA insert upstream of the fibre gene by Cre-lox recombination



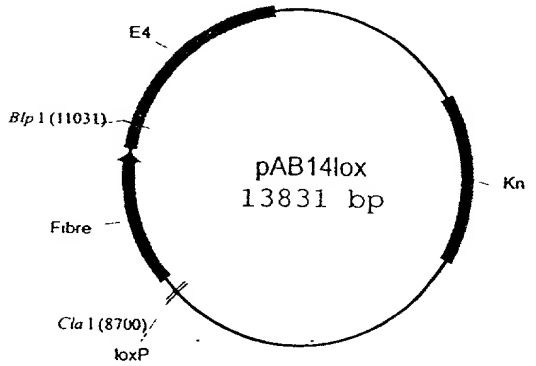
RECOMBINANT VIRUS CONTAINING AN INSERT OF FOREIGN DNA UPSTREAM OF THE FIBRE GENE

Figure 13

CONSTRUCTION OF pAB14FL0X FOR ISOLATION OF AN AD VIRUS WITH A FLOXED FIBRE GENE



Insert loxP linker
SEQ. ID. NO.: 5
and SEQ. ID. NO.: 6
into Xba I site



Insert loxP linker
SEQ. ID. NO.: 7
and SEQ. ID. NO.: 8
into Blp I site

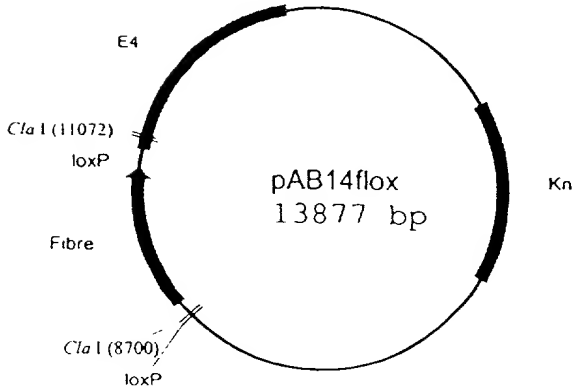
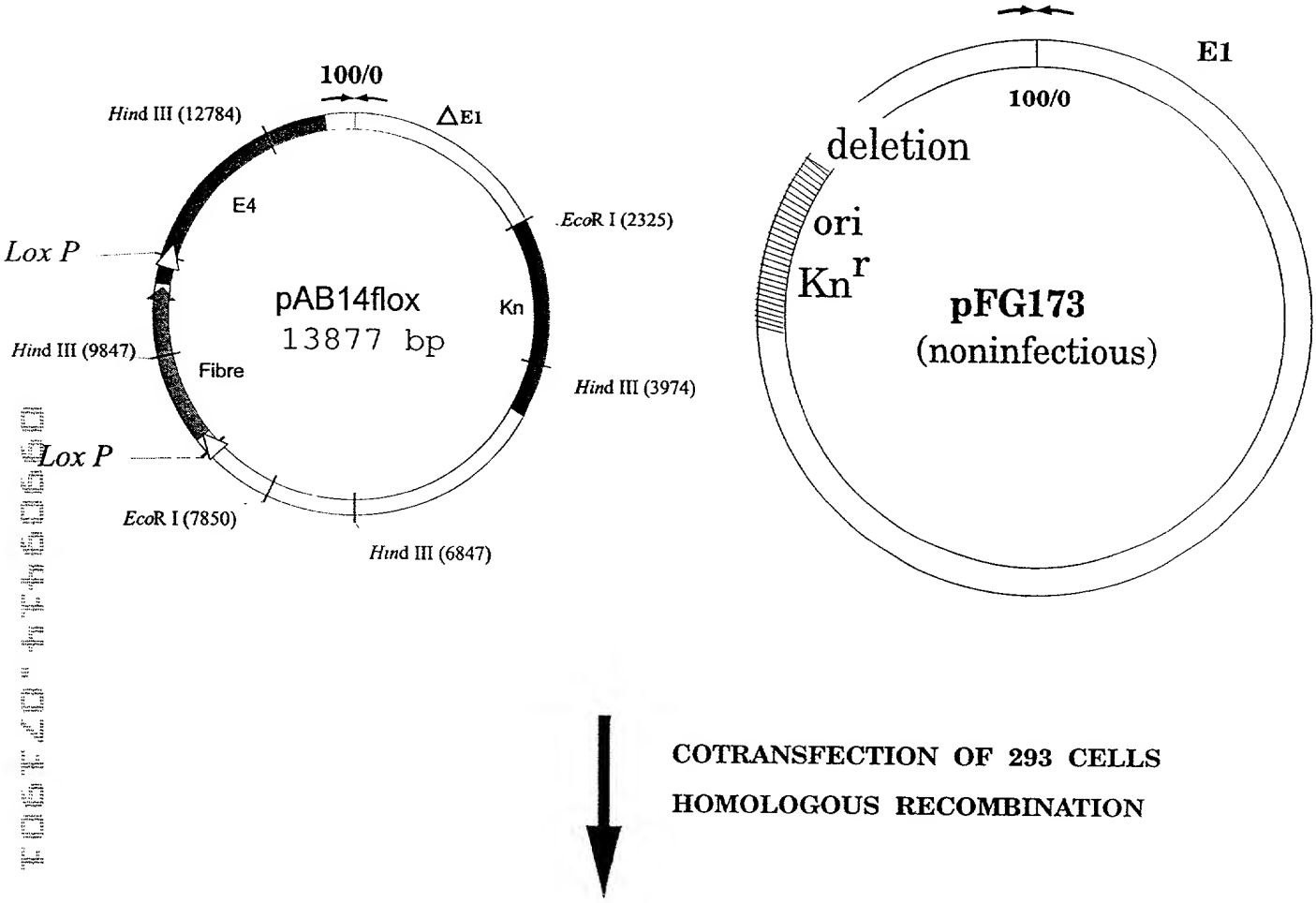


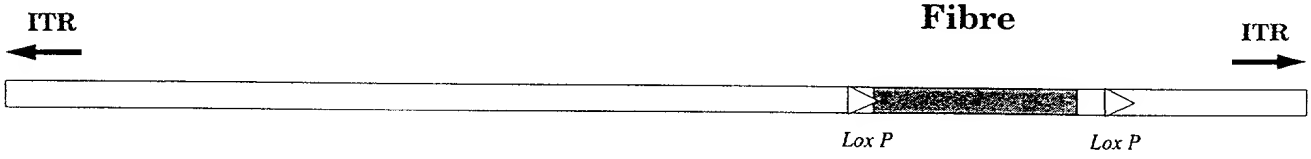
Figure 14

Isolation of a virus containing a fibre gene with flanking lox P sites.



COTRANSFECTION OF 293 CELLS

HOMOLOGOUS RECOMBINATION



+

NONDEFECTIVE (E1) VIRUS (ADFLOXFIBRE) CONTAINING A FLOXED FIBRE GENE

Figure 15